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IMMUNOASSAY OF MOUSE IMMUNOGLOBULIN G BY A LIGHT-ADDRESSABLE POTENTIOMETRIC SENSOR

by

H. Gail Thompson and William E. Lee

PCN 351SQ

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Immunoassay of Mouse Immunoglobulin G by a Light-Addressable Potentiometric Sensor

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Acknowledgements

We would like to thank J. P. Wong, R. E. Fulton and T. V. Jacobson for their helpful discussions during the course of this work and B. Thomas and G. Bhogal for the design and modification of the pipettor.

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ABSTRACT

A sensitive enzyme immunoassay for the quantitation of mouse immunoglobulin G (mIgG) was developed using a light-addressable potentiometric (LAP) sensor as the detection system. The assay was carried out on nitrocellulose membrane filters and used sandwich-format one-step incubations of antigens and antibodies. In addition, the assay employed a high affinity biotin-streptavidin interaction to capture the immunocomplexes onto nitrocellulose membranes, and a urease-conjugated anti-mouse IgF detector antibody. The lower limits of detection of mIgG by this assay were 300, 100, and 10 pg per well, respectively, for 1, 5, and 60 min incubations. The time required for the assay was the incubation time plus 4-5 min, in total for the mixing, washing and detection procedures.

RÉSUMÉ

Un test immuno-enzymatique sensible, utilisant un titrimètre adressable par la lumière (TAL) comme système de détection, a été mis au point pour quantifier l'immunoglobuline G de souris. Le test a été effectué avec des filtres à membranes de nitrocellulose; l'incubation des antigènes et des anticorps a été faite en une étape avec une méthode de type «sandwich». Le test utilise, de plus, la forte affinité de l'avidine pour la biotine afin de capturer les complexes immuns sur des membranes de nitrocellulose ainsi qu'un anticorps détecteur, un anti IgG de souris conjugué à l'uréase. Les limites inférieures de détection de l'IgG de souris obtenues pour 1, 5 et 60 min d'incubation ont été de 300, 100 et 10 pg par puits respectivement. Le test a été réalisé en 4 à 5 minutes (plus le temps d'incubation), soit le temps nécessaire pour les opérations de mélange, de lavage et de détection.

INTRODUCTION

The role of silicon based semiconductor detection devices is increasingly important in biosensor technology. Semiconductors provide a number of advantageous qualities such as high sensitivity, low power requirements, durability, multiplicity of measurement sites and the capacity for miniaturization (1). When coupled with advances in enzyme immunoassay technology, silicon based detectors provide a powerful tool for immunochemical assays.

The BioChemical Detector (BCD) project is a trinational research program involving the United Kingdom, United States and Canada. The goal of the program is to develop a lightweight detector capable of responding rapidly, within two to five minutes, to a range of chemical and biological agents. The biological agents will include proteins, viruses and bacteria. The biosensor for the BCD was designed under contract with the Chemical Research Development and Engineering Centre of the U.S. Army (Aberdeen, MD) by Environmental Technologies Group (Baltimore, MD). The technology chosen for the biological agent detector is based on a light-addressable potentiometric (LAP) sensor, a silicon semiconducting device (2). This new technology was developed by Molecular Devices Corporation (Menlo Park, CA).

As part of Canada's contribution to the BCD program, the task of developing a standard antigen assay for the LAP sensor was undertaken. The assay would serve as an international standard for comparison of work done in the laboratories of the three nations. It was designed to use reagents that are commerically available and readily obtainable from international suppliers. The antigen chosen for the standard assay was mouse immunoglobulin G (mIgG). This protein, as well as the conjugated antibodies directed against it that were required for the assay, were readily available at moderate cost. In addition,

mIgG served as a prototype for other agents associated with the BCD. Because the LAP sensor technology was essentially new and there was no body of reference literature available to the BCD research program, this work provided a vehicle for the study of LAP sensor technology and the evaluation of its application to the detection requirements of the BCD program.

The enzyme immunoassays carried out in this work involved the formation of antibody-antigen complexes in a liquid phase followed by streptavidin-biotin mediated filtration onto biotinylated nitrocellulose membrane (3). The immobilized enzyme, urease, associated with the antibody-antigen immunocomplex was detected by the LAP sensor, specifically designed to accommodate the membrane.

The purpose of this work was to examine the response of the LAP sensor to the standard antigen, mIgG, and to determine the effects of incubation time and temperature on the LAP sensor response. The aspects of the response examined were the lower limit of detection (LOD) and sensitivity. These concepts when applied to a quantitative assay are a reflection of the ability of the assay to detect the analyte, but they are distinct properties and have different meanings (4a). Sensitivity is defined by the dose-response curve of the assay. It is the change in the response (dR) per unit amount of analyte (dA) and is equal to dR/dA, the slope of the dose-response curve. In a given assay dR/dA is not by definition a constant. LOD refers to the least amount of analyte which produces a response above a preset background or minimum level. In this work the term, detectability (the ability to detect lesser amounts of analyte), is defined as the reciprocal of LOD and is used in a comparative context. For example, comparing two assays A and B, if the LOD of assay A is one-half that of B, then the detectability of A is twice that of B.

This work describes the quantitative detection of picogram amounts of antigen using filtration capture followed by potentiometric sensing. The relevance of incubation time and temperature of the immunoassay to the design of the BCD is presented.

MATERIALS AND METHODS

Reagents

Mouse immunoglobulin G, biotinylated anti-mouse IgG (goat), urease-conjugated anti-mouse IgG (goat), bovine serum albumin (BSA), sodium chloride, sodium dihydrogen phosphate, Tween 20, Triton X-100, phosphate-buffered saline and urea were obtained from Sigma Chemical Co. (St Louis, MO) and used without any further purification. Streptavidin was obtained from Scripps Laboratories (San Diego, CA). It was reconstituted in distilled water to yield a stock concentration of 10 mg/ml and stored at -20°C in sealed vials.

Wash solution was prepared from 150 mM NaCl, 10 mM phosphate buffer pH 6.5 plus 0.2% Tween 20 detergent. The dilution buffer was the wash solution titrated to pH 7.0, containing 1% (w/v) BSA and 0.25% (w/v) Triton X-100. The substrate solution for the enzyme assay was wash solution containing 100 mM urea.

Apparatus

The apparatus for these assays was a commerically available LAP sensor, marketed under the name Threshold Unit™ by Molecular Devices Corp. (Menlo Park, CA). The instrument was controlled by an IBM PS/2 model 30 microcomputer and custom designed software supplied by Molecular Devices Corp. The design of the Threshold Unit allowed eight samples to be tested simultaneously. Nitrocellulose membranes, embedded with biotin and blocked with BSA, were purchased from Molecular Devices Corp.

Immunoassay Procedures

Mouse IgG was prepared as a stock solution (2.5 mg/ml) in phosphate-buffered saline and stored at -20°C in sealed vials. This solution was diluted with dilution buffer to produce the required standards for calibration curves and the samples for quantitation. A reagent solution was prepared from 10 μl urease-conjugated anti-mouse IgG (0.88 mg/ml), 22 μl biotinylated anti-mouse IgG (0.35 mg/ml) and 9 μl streptavidin (10 mg/ml) in 10 ml of dilution buffer. The reagent solution was allowed to stand at room temperature for two to three hours prior to use.

Figure 1 provides a schematic representation of the sandwich immunoassay for mIgG. A volume of 150 μl of reagent solution was added to 100 μl of diluted mIgG sample. The reagents and sample were mixed thoroughly for 20 sec and incubated for the required length of time. At the end of the incubation period, a portion of the incubated sample-reagent mixture, 150 μl, was delivered to a well of the filter assembly of the Threshold Unit. This aliquot of 150 μl contained the equivalent of 60 μl of mIgG sample and 90 μl of reagent solution.

Filtration Capture and Potentiometric Sensing

The sample-reagent mixture was filtered through the biotinylated nitrocellulose membrane at 250 μl per min. The streptavidin in the reagent solution acted as the bridge between the antigen-biotinylated antibody complexes and the immobilized biotin on the nitrocellulose membrane. The membrane was then washed with 0.5 ml of wash solution and filtration rate was increased to 750 μl per min.

The membrane stick containing immobilized immunocomplexes was removed from the filter assembly and inserted into the reader compartment of the Threshold Unit which contained the LAP sensor and the urea substrate solution. A plunger pressed the membrane against the surface of the silicon the sensor. The instrument was designed so that the spots on the surface of the membrane which contained immobilized immunocomplexes, aligned with the pH sensitive measurement sites on the surface of LAP sensor. At the surface of the sensor, the hydrolysis of urea to carbon dioxide and ammonia caused an increase in the pH which was detected as a change in the surface electropotential (2). The data was recorded and stored on the microprocessor using the custom designed software. The rate of change of pH at the surface of the silicon sensor was monitored by the rate of change with respect to time of the surface potential as μv/sec.

RESULTS

Detection of mIgG on the LAP Sensor

The lower limits of detection of the LAP sensor for mIgG were determined for incubation times of one, five and sixty minutes at room temperature. Figure 2 is a standard curve for an incubation time of one minute. The standards ranged from 0.2 to 5 ng per well. Each point on the standard curve is the mean of three consecutive runs. The data, μv/sec versus ng per well, was represented well by a linear plot. The errors associated with the individual points on the calibration curve were about 12%. The LOD, taken to be the intersection of the calibration curve with the mean background (output of the LAP sensor for reagents only, with zero mIgG antigen) plus two standard deviations (SD), was 300 pg per well.

The mIgG assays were carried out for a five-minute incubation using standards ranging from 50 to 1000 ng per well. Using the criterion as above, the LOD was determined to be about 100 pg (Figure 3). For a sixty minute incubation with standards ranging from 6 to 75 pg, the LOD was determined to be about 10 pg per well (Figure 4). This value of LOD compares well with a LAP sensor assay for the protein, human chorionic gonadotropin (hCG) (5). There, a LOD of 10 pg was obtained in a one-step sandwich format assay. In terms of detectability, the assays having five and sixty minute incubations displayed an increase of 3- and 30-fold, respectively, over the assay of the one minute incubation. The increase in the sensitivity of the five and sixty minute assays over the one minute assay, as determined from the slopes of the calibration curves (Figures 2-4), was about 5- and 40-fold, repectively.

Various amounts of mIgG were presented to the LAP sensor and assayed (n = 3). The recovered (measured) values, calculated from standard curves, were within 10% of the actual amount of mIgG added (Table I). Coefficients of variation (SD/mean) were 13% or less.

In order to examine the effect of some interferents on the performance of the LAP sensor, assays of mouse IgG were carried out in the presence of horse serum and two mammalian immunoglobulin G proteins, rabbit and rat. The samples with horse serum as the interferent contained 50% (v/v) horse serum. From the data shown in Table II, there was no appreciable effect on the assay when 50% horse serum was added. Rabbit IgG at one thousand fold excess showed negligible effect on the quantitative assay. For rat IgG there was a significant amount of interference, to be expected due to cross reactivity. At 10-fold excess of rat IgG the sensor output was enhanced by a factor of 1.4. Greater excesses of rat IgG resulted in larger enhancements of the output; for 1000-fold excess the enhancement was about 5-fold.

The LAP sensor assays typically contained 0.13, 0.08 and 1.3 μg per well of urease-conjugated antibody, biotinylated antibody and streptavidin. These amounts provided a molar excess of the reagent antibodies over the amount of mIgG present in the samples used in the standard curves and quantitations, as well as a molar excess of streptavidin over the biotinylated antibody (in this work about 40:1). Empirically it was found (data not shown) that a molar excess of the biotinylated antibody over the urease-conjugated antibody (in this work about 2.5:1) produced favourable results. The concentrations of the components of the reagent solution were adjusted to produce backgrounds in the range of 300 - 400 μv/sec. These provided the most reproducible results and the least amount of scattering of the data for samples of mIgG ranging from 5 pg to 20 ng and incubation times of one to sixty minutes.

Effects of Incubation Time and Temperature on the Response of the LAP Sensor

The output of the LAP sensor (μv/sec) is dependent upon the number of antibody-antigen immunocomplexes immobilized onto the membrane during the filtration capture process as depicted in Figure 1. Since the efficiency of the filtration capture of streptavidin onto biotinylated nitrocellulose is high, about 95% under the conditions of these assays (5), the total number of immunocomplexes immobilized and hence the sensor output will be a function of the number formed during the single-step incubation.

Fixed amounts of mIgG were incubated with the reagent solutions for times ranging from one to sixty minutes. From the raw data, signal-to-noise ratios (S/N) were calculated and plotted versus incubation time (Figure 5). The noise component (N) of the ratio was the background of the assay, i.e., the sensor output for reagents only, with no mIgG. The signal component (S) was the sensor output for a sample containing 5 ng mIgG, less the

noise component. Each point in Figure 5 represents 16 determinations of signal and of noise. It is apparent that with increasing incubation time there was an increase in S/N. For incubations of 1, 5 and 60 minutes, S/N was 0.5, 2.6 and 17, repectively. The relative increase in S/N of the 5 and 60 minute incubations over the 1 min incubation was 5- and 34-fold. It is also apparent that assays having short incubation times produced only a small fraction of the total potential signal. Both the detectability and S/N of the LAP sensor increased with increasing incubation time and the correlation between these two properties was strong (r^2 = 0.9988, Figure 6).

We can compare the results of the LAP sensor assay for Newcastle Disease Virus (NDV) (6) with the results for mIgG. Similar to the mIgG results, the correlation between detectability and S/N for NDV was strong (r^2 = 0.999). However the response of S/N to the length of the incubation time for the individual assays of mIgG and NDV was different (Figure 7); S/N for mIgG increased at a greater rate.

Standard curves of mIgG were run for incubation times of 1, 5 and 60 minutes at temperatures of 23 and 37°C. For incubations of 1 and 5 minutes there was a marked difference between the results at the two temperatures (Figures 8 and 9). The slopes of the standard curves, and hence the sensitivities of the assays were greater by a factor of about 2.5 at the higher temperature. For the 60 minute assay the difference between the standard curves at 23 and 37°C was minimal (Figure 10).

DISCUSSION

The increase of S/N with incubation time (Figure 5) reflects the initial formation process of the antibody-antigen complexes which, in turn, depends upon the association rate constant, k_a. For a large number of antigen-antibody systems, the value of k_a is nearly equal to the diffusion limit in aqueous media of the reacting species (4b). Since the dif-

fusional rate constants have an inverse dependency upon molecular weight (7), i.e., at a given temperature larger particles move slower, then as the molecular weight of the antigen increases, k_a decreases (8). From an estimate of the shape and size of NDV, irregular spheroid 150 nm in diameter (9), we calculate the molecular weight to be approximately 1.4 x 10^9 g, which is about four orders of magnitude larger than the molecular weight of IgG, about 150,000 (10). The behaviour of NDV and mIgG as antigens (Figure 7) is consistent with this analysis, the increase in S/N with increasing incubation time is greater for the smaller antigen, mIgG. In general, the law of mass action will make it more difficult to detect relatively larger sized antigens in a rapid assay format, a format where the equilibrium is neither reached nor approached. On a gram-to-gram basis, there are fewer of the larger sized antigens to detect and k_a for the larger antigens is less due to the slower diffusion through the aqueous medium.

The effect of incubation temperature on the output of the LAP sensor (Figures 8,9) suggests that kinetic aspects of the antigen-antibody interactions are major factors contributing to the diminished sensitivity at short incubation times. That is, the limiting factor is the association of the antibody-antigen complex. The short reaction time does not allow significant dissociation of the complex to occur and hence the concentration of antibody-antigen complex is most strongly dependent upon k_a and not the equilibrium constant. Increasing the temperature will result in an increase in k_a. The dependence of k_a of antibody-antigen interactions on temperature derives from Arrhenius rate theory (11):

$$k = A\, e^{-B/T}$$

where k is the rate constant, T is the absolute temperature, and A and B are constants particular to the chemical system.

For longer incubation times, as the system approaches equilibrium, the dissociation rate constant of the complex, k_d, has a greater effect on the number of complexes in the reaction mixture. The increased temperature which enhances k_a also enhances k_d and thus equilibrium constants (note: equilibrium constant, $K_a = k_a/k_d$) are not as sensitive to temperature as the component rate constants. This is illustrated by Figure 10. The standard curves obtained from sixty minute incubations were essentially the same for 23 and 37°C.

The goal of the BCD program is to develop a rapid immunoassay system, one in which incubations times are limited to one to two minutes. The conclusion of this work is that in a nonequilibrium time frame, the characteristics of the LAP sensor assay, these being LOD, sensitivity and S/N, are a function of incubation time and temperature. The assay protocol of the BCD will employ one-step sandwich immunoassays as used in this work. However, it will not use standard curves to detect the presence of antigen, rather the output from a test site on the LAP sensor will be compared to a reference. Thus, for the BCD, in addition to the concentration of antigen present in the test sample, the LAP sensor output will also be dependent upon incubation time and internal temperature. Future design considerations of the BCD should address these factors.

At room temperature the LAP sensor displayed a high level of stability and reproducibility both in this work and the assay of NDV (6). The reproducibility was high enough to allow the averaging of consecutively run standard curves. The standard errors of the individual data points were about 12%. The day-to-day consistency of the LAP sensor assays was also good. LOD values obtained for a given incubation time and temperature did not vary significantly when the assays were performed on different days using freshly prepared antibody and antigen solutions.

On the calibration curves (Figures 2-4), the ratio of the sensor output to mean background (s/b) at the LOD of the assays was about 1.10 (i.e., 1.11, 1.10, 1.09 for 1, 5, 60 min incubations, respectively). Even though the length of the incubation time increased from

one to sixty minutes, s/b at the LOD remained constant. In the LAP sensor assay of NDV with incubation times ranging from one to sixty minutes, s/b values at the LOD were also about equal to 1.1 (6). One of the principle reasons that s/b values at the LOD were consistent for both the mIgG and NDV assays was that the background output of the LAP sensor was relatively constant; standard deviations on the background were 10-12% or less for an assay conducted on a particular day. In a regime of constant temperature and incubation time, reproducible qualitative detection of an antigen, above a limiting LOD level, as determined from the ratio of the output of the test site to a reference site, is feasible for the LAP sensor.

The characteristics of the assays, S/N, LOD and sensitivity, although related, are nonetheless distinct. From the work to date we have found that the correlation among these properties is strong in the LAP sensor assays for both mIgG and NDV. Thus the response of characteristic properties of the a LAP sensor assay, i.e., LOD and sensitivity, to changes in incubation time and temperature can be estimated with confidence from the variation in S/N for a fixed concentration of antigen.

CONCLUSIONS

In summary, the work presented here has described a one-step sandwich immunoassay of mIgG using a LAP sensor. A LOD of 10 pg with two standard deviations separation above background was obtained for incubations of sixty minutes. The overall time required for the assay was the incubation time plus an additional four to five minutes for filtration capture, washing and potentiometric sensing. The assay has a high specificity for the murine IgG. A 1000-fold ratio (w/w) of contaminant, rabbit IgG, to mouse IgG had negli-

gible effect on the output signal. Similar results were observed when the assays were carried out in 50% horse serum. The salts and organic material introduced to the assay by the horse serum had no measurable effect. These results suggest that the LAP sensor assays were not very sensitive to biological interferents although a more detailed study will be undertaken with the BCD. The stability and reproducibility of the LAP sensor assay in run-to-run and day-to-day assays was good. The results of this study would indicate that qualitative assays of biological materials by a LAP sensor are feasible in a format that is compatible to the design of the BCD. The mIgG asay developed here is suitable to be used as an interlaboratory standard.

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Figure Captions

Figure 1. The reaction scheme of the LAP sensor.

Figure 2. Calibration curve of mouse IgG for a 1 minute assay. Each data point is the average of three determinations. The bars are ± 1 standard deviation. The LOD was taken to be the intersection of the calibration curve (linear regression, y = 333 + 78.9x, r^2 = 0.978) and the background plus two SD.

Figure 3. Calibration curve of mouse IgG for a 5 minute assay. Each data point is the average of three determinations. The bars are ± 1 standard deviation. The LOD was taken to be the intersection of the calibration curve (linear regression, y = 425 + 0.360x, r^2 = 0.989) and the background plus two SD.

Figure 4. Calibration curve of mouse IgG for a 60 minute assay. Each data point is the average of three determinations. The bars are ± 1 standard deviation. The LOD was taken to be the intersection of the calibration curve (linear regression, y = 399 + 3.26x r^2 = 0.991) and the background plus two SD.

Figure 5. The signal-to-background ratio versus incubation. Each data point is the quotient of the signal (avg., n = 16) and the background (avg., n = 16). The bars are ± sums of 1 SD of signal and 1 SD of background.

Figure 6. Correlation of the relative detectability and signal-to-noise ratio for the assay of mIgG. The values of detectability and S/N given are normalized to those of the 1 min assay.

Figure 7. A logarithmic plot of S/N versus incubation time for mIgG and NDV (Ref. 11)

Figure 8. 1 min incubation of mIgG at 23°C and 37°C. The data were fitted by a linear regression, 23°C, $y = 352 + 10.5x$, $r^2 = 0.967$; 37°C, $y = 404 + 25.0x$, $r^2 = 0.996$.

Figure 9. 5 min incubation of mIgG at 23°C and 37°. The data were fitted by a linear regression, 23°C, $y = 317 + 43.6x$, $r^2 = 0.992$; 37°C, $y = 333 + 107x$, $r^2 = 0.993$.

Figure 10. 60 min incubation of mIgG at 23°C and 37°.

Table I

Quantitation of mIgG on the LAP Sensor

mIgG added (ng per well)	mIgG recovered (ng per well)	% Recovery	%CV
0.125	0.126	101	7.9
0.500	0.515	103	3.5
6.25	6.30	101	13
10.0	10.0	100	11
12.5	11.3	90	11

Precision and accuracy of mIgG assay. Samples were presented to the LAP sensor and assayed (n=3). The recovered (measured) values and coefficients of variation were determined from standard curves.

Table II

Interferents for LAP Sensor Assay of mIgG

Interferent	sensor output w/o interferent μv/sec (SD)	sensor output with interferent μv/sec (SD)	relative increase in output
horse serum[a]	999 (59)	951 (76)	nil
rabbit IgG[b] (1000/1,w/w)	544 (38)	537 (72)	nil
rat IgG[b] (100/1,w/w)	430 (40)	620 (80)	1.4
rat IgG[b] (100/1,w/w)	671 (56)	1409 (52)	2.1
rat IgG[b] (1000/1,w/w)	499 (28)	2342 (204)	4.7

[a]the sample was 15 ng per well mIgG (n=4) incubated with immunoreagents for one minute at room temperature in a solution consisting of 50% (v/v) horse serum.

[b]the sample was 8.5 ng per well mIgG (n=4) incubated with immunoreagents and interferent (ng interferent/ng mIgG) for one minute at room temperature.

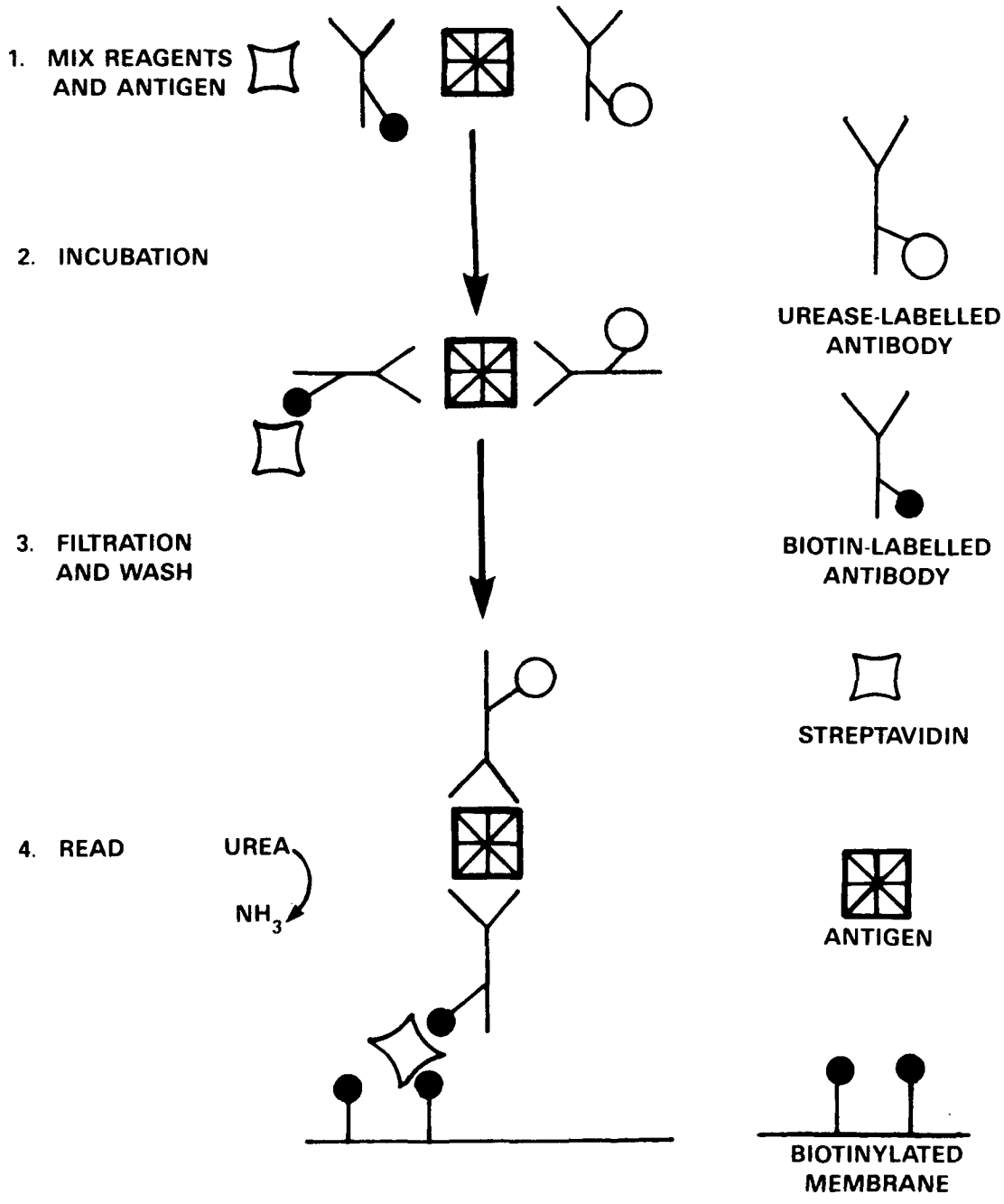

Figure 1

THE REACTION SCHEME OF THE LAP SENSOR.

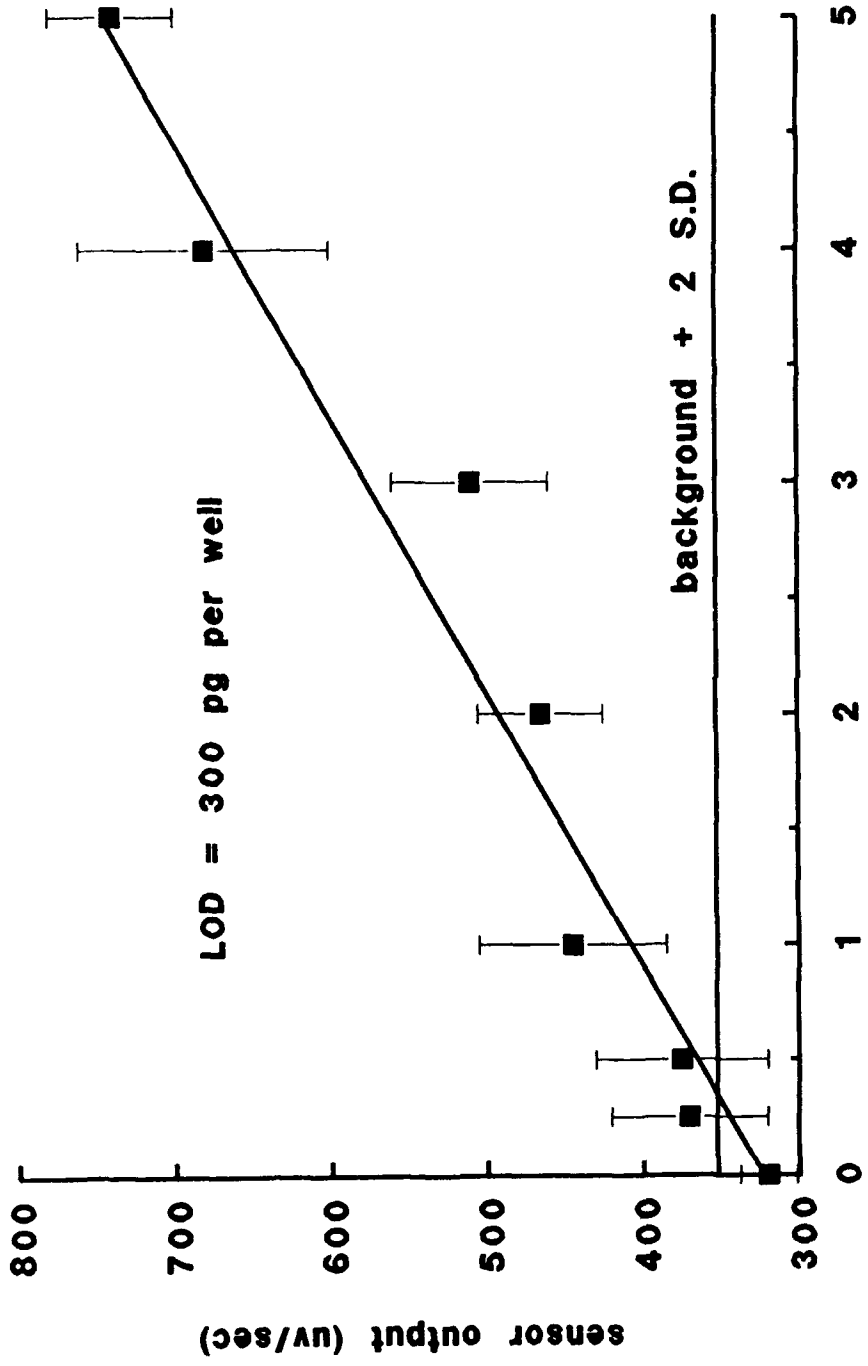

Figure 2

CALIBRATION CURVE OF MOUSE IgG FOR 1 MINUTE ASSAY. EACH DATA POINT IS THE MEAN OF THREE DETERMINATIONS. THE BARS ARE ±1 STANDARD DEVIATON. THE LOD WAS TAKEN TO BE THE INTERSECTION OF THE CALIBRATION CURVE (LINEAR REGRESSION, $y = 333 + 78.9x$, $r^2 = 0.978$) AND THE BACKGROUND PLUS 2 SD.

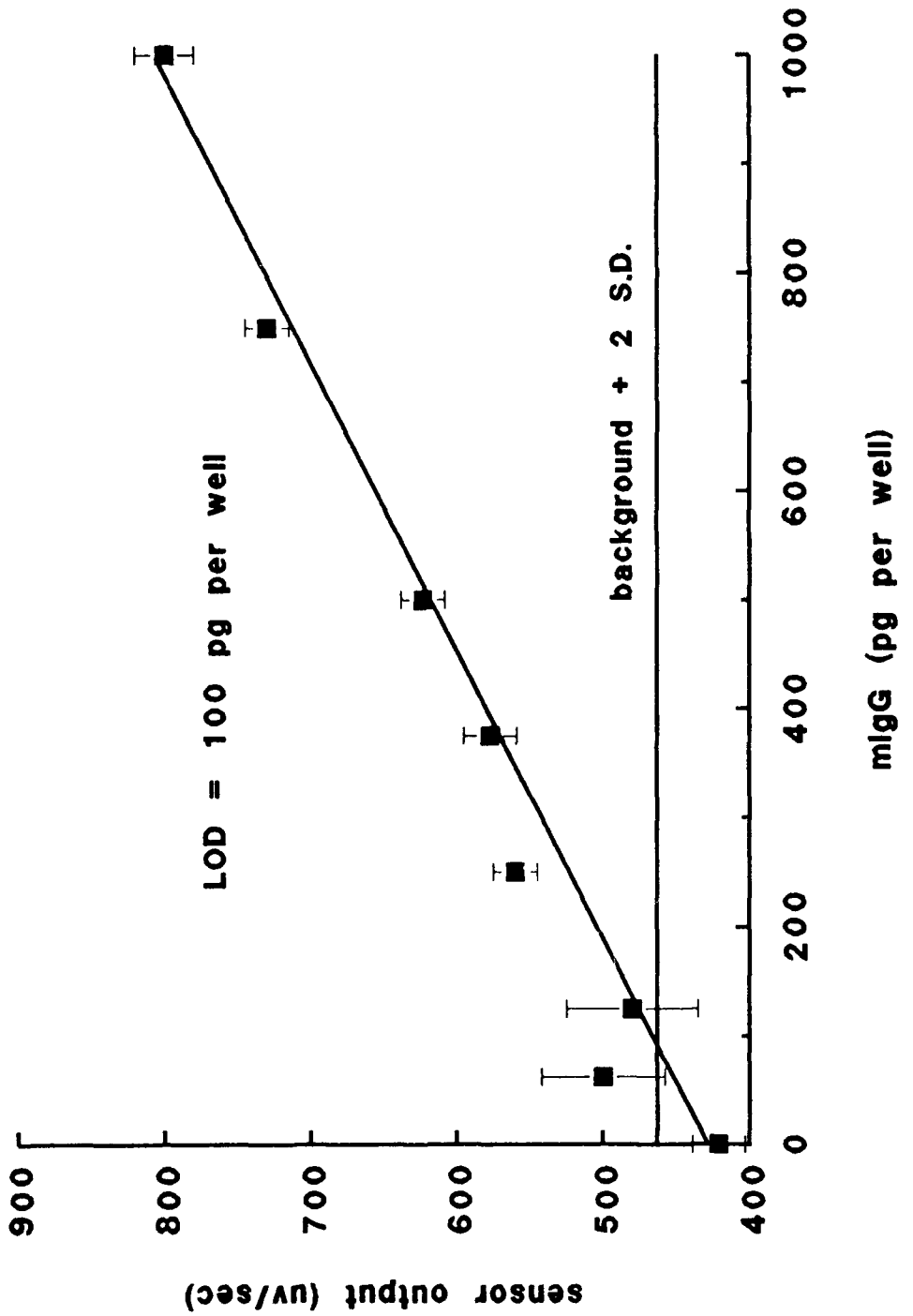

Figure 3

CALIBRATION CURVE OF MOUSE IgG FOR A 5 MINUTE ASSAY. EACH DATA POINT IS THE MEAN OF THREE DETERMINATIONS. THE BARS ARE ±1 STANDARD DEVIATION. THE LOD WAS TAKEN TO BE THE INTERSECTION OF THE CALIBRATION CURVE (LINEAR REGRESSION, $y = 425 + 0.360x$, $r^2 = 0.989$) AND THE BACKGROUND PLUS 2 SD.

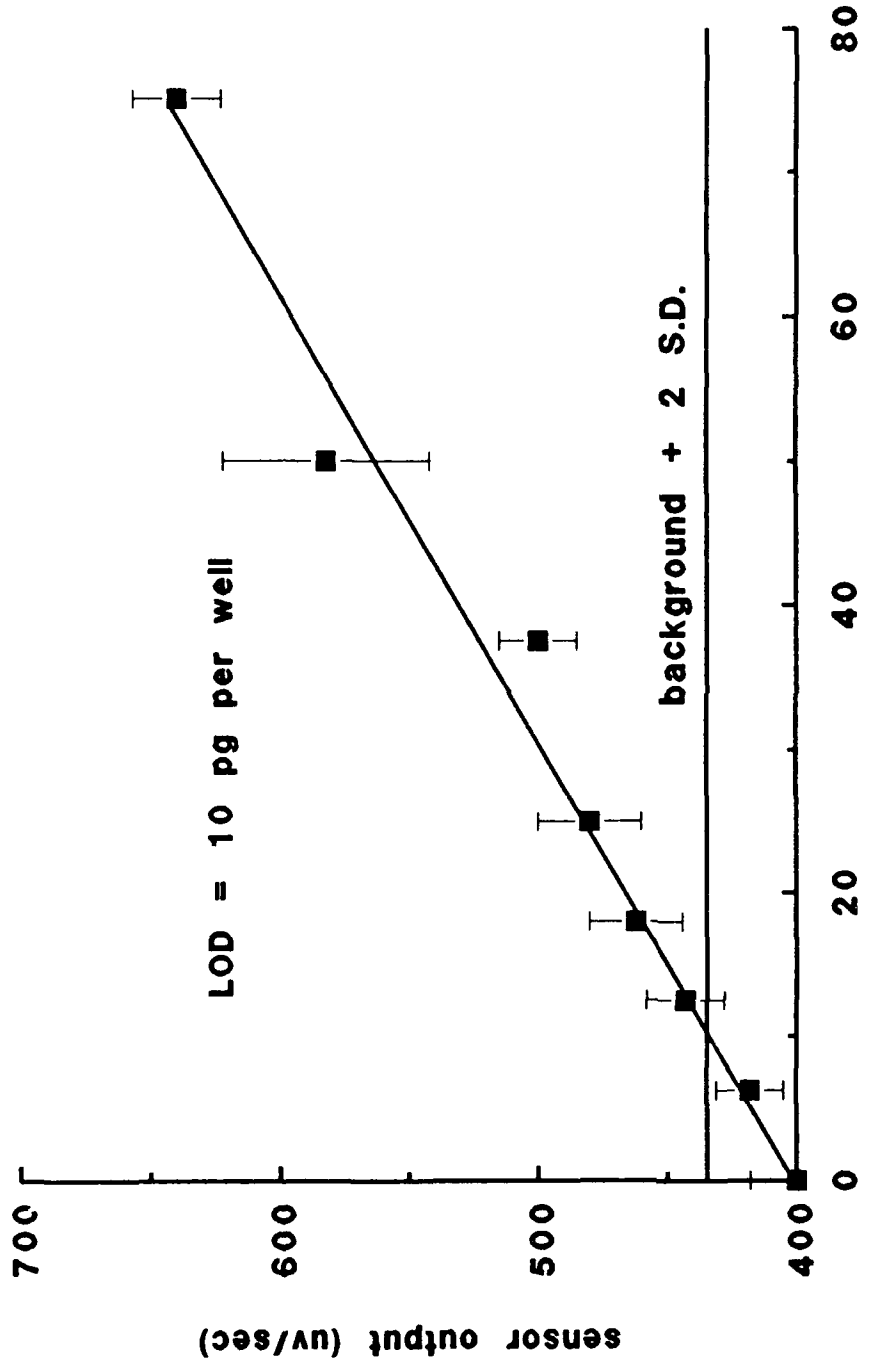

Figure 4

CALIBRATION CURVE OF MOUSE IgG FOR A 60 MINUTE ASSAY. EACH DATA POINT IS THE MEAN OF THREE DETERMINATIONS. THE BARS ARE ±1 STANDARD DEVIATION. THE LOD WAS TAKEN TO BE THE INTERSECTION OF THE CALIBRATION CURVE (LINEAR REGRESSION, $y = 399 + 3.26x$ $r^2 = 0.991$) AND THE BACKGROUND PLUS 2 SD.

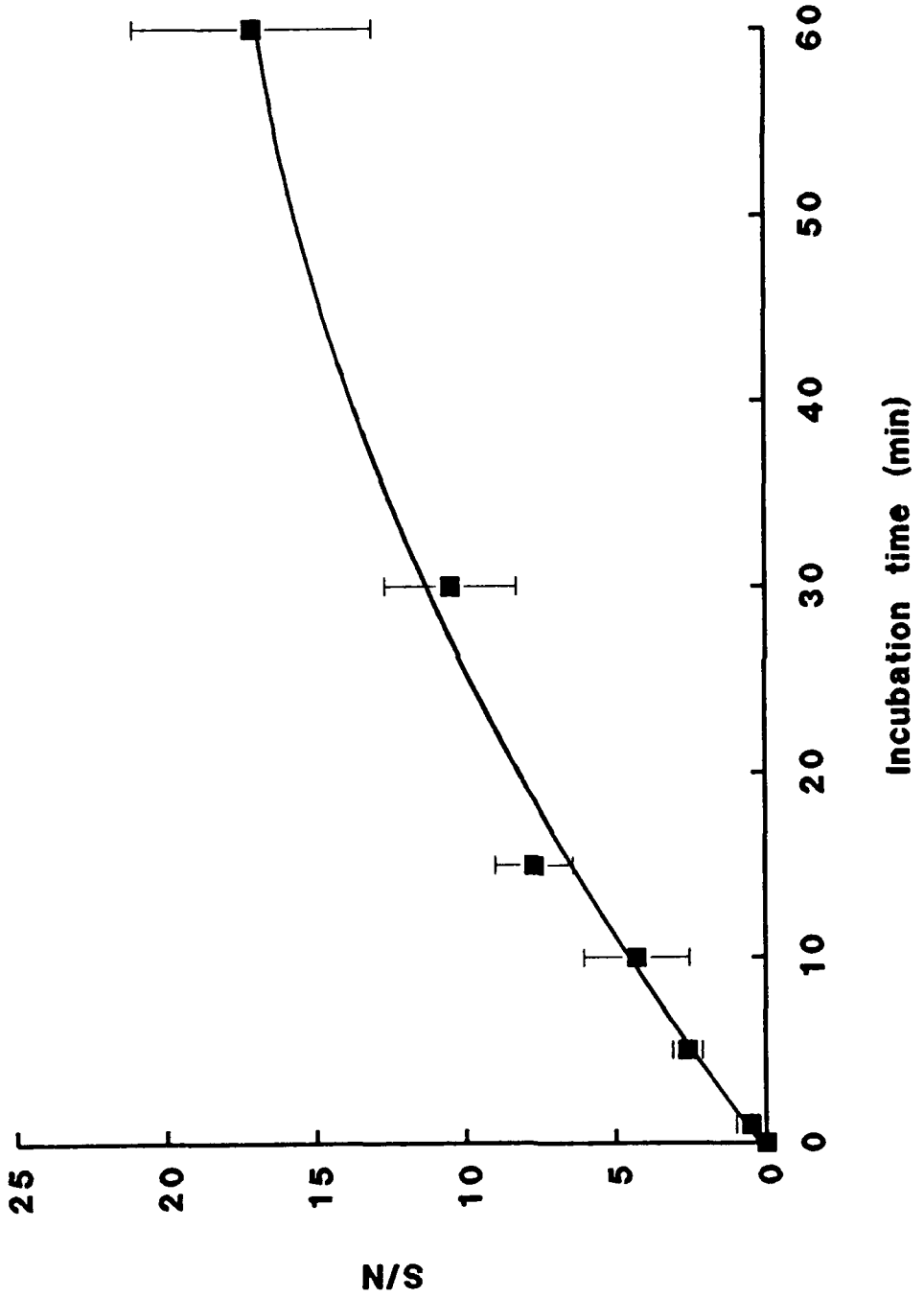

Figure 5

THE SIGNAL-TO-BACKGROUND RATIO VERSUS INCUBATION TIME. EACH DATA POINT IS THE QUOTIENT OF THE SIGNAL (MEAN, n = 16) AND THE BACKGROUND (MEAN, n = 16). THE BARS ARE ± SUMS OF 1 SD OF SIGNAL AND 1 SD OF BACKGROUND.

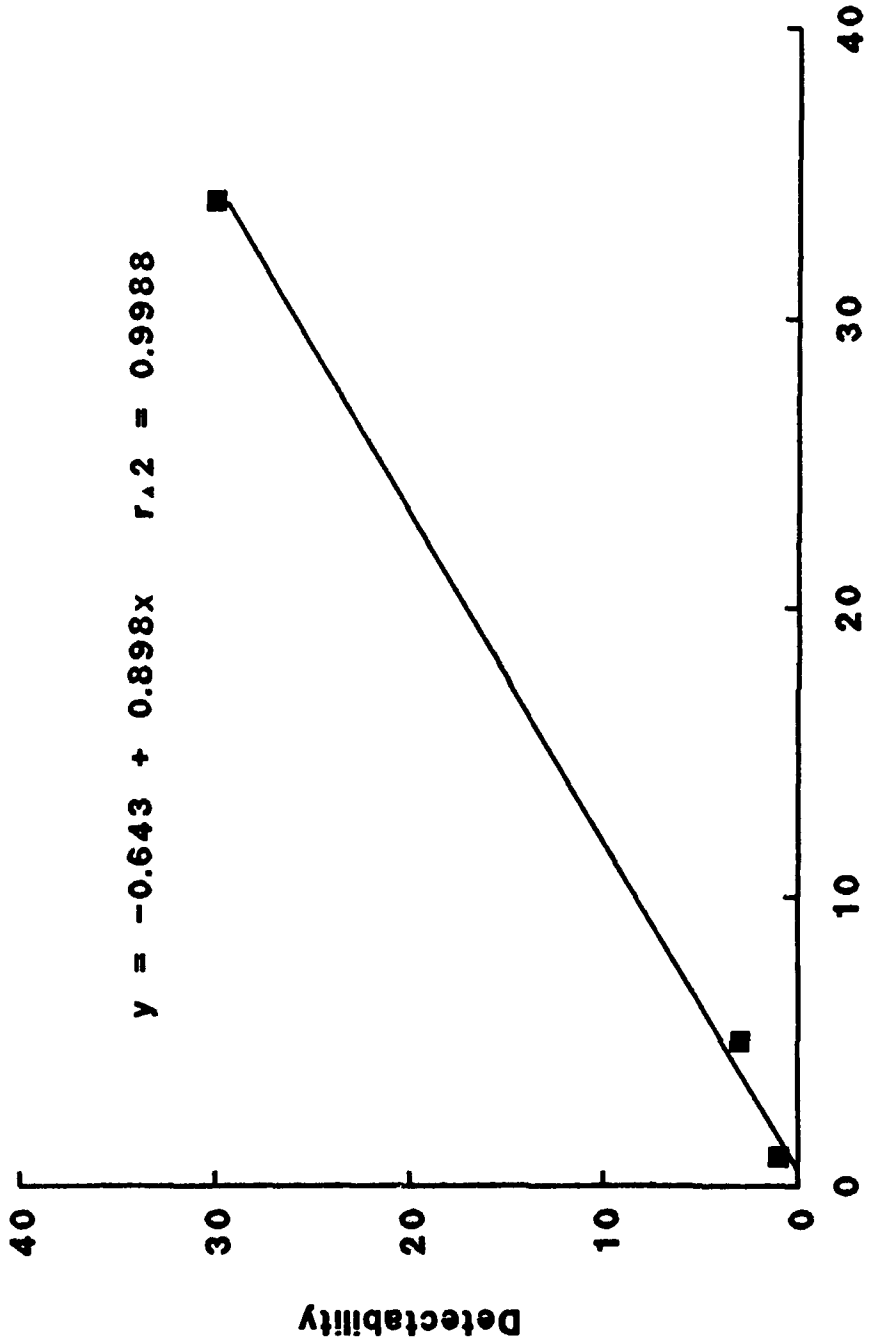

Figure 6

CORRELATION OF THE RELATIVE DETECTABILITY AND SIGNAL-TO-NOISE RATIO FOR THE ASSAY OF mIgG. THE VALUES OF DETECTABILITY AND S/N GIVEN ARE NORMALIZED TO THOSE OF THE 1 MINUTE ASSAY.

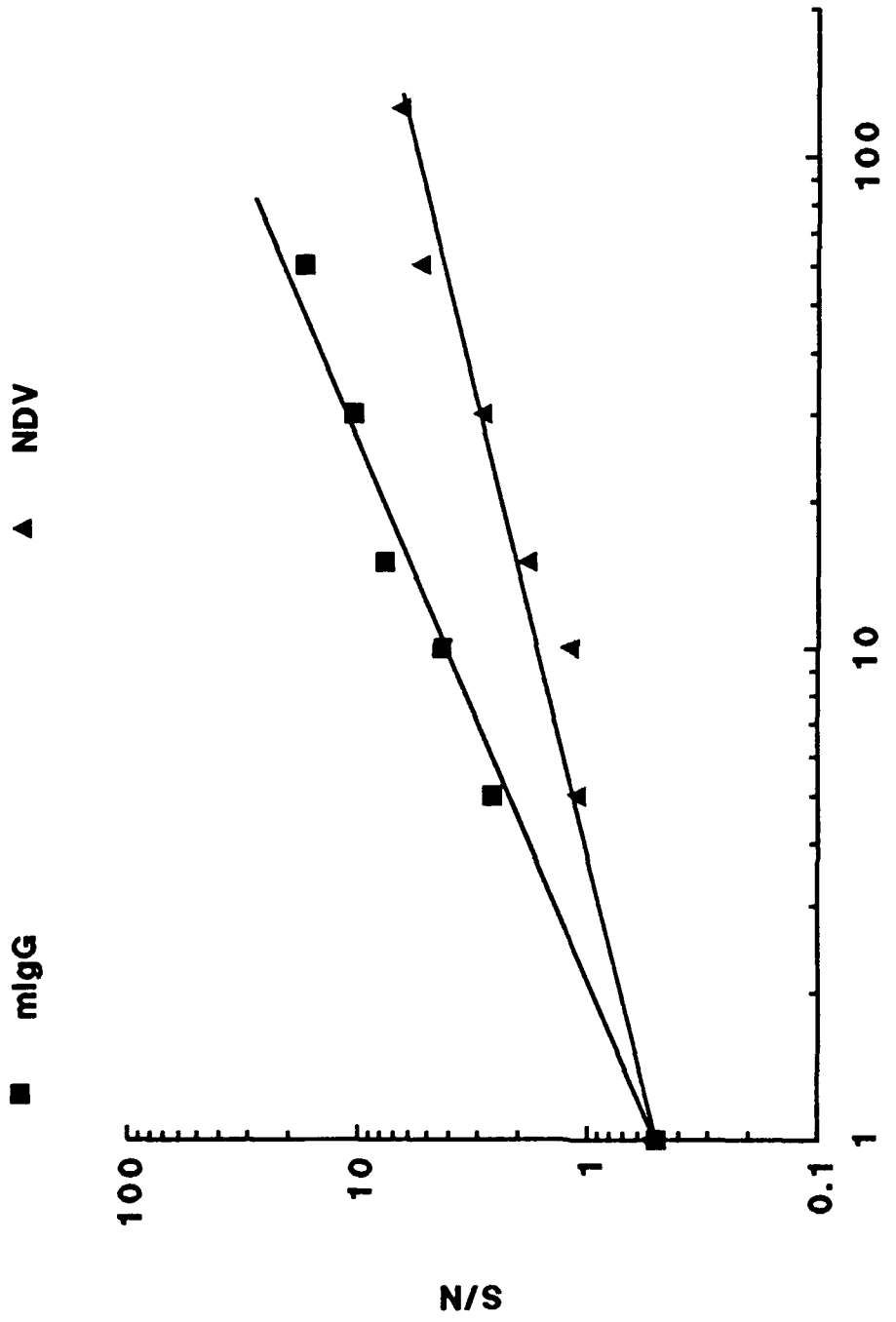

Figure 7

A LOGARITHMIC PLOT OF S/N VERSUS INCUBATION TIME FOR mIgG AND NDV (REF. 11).

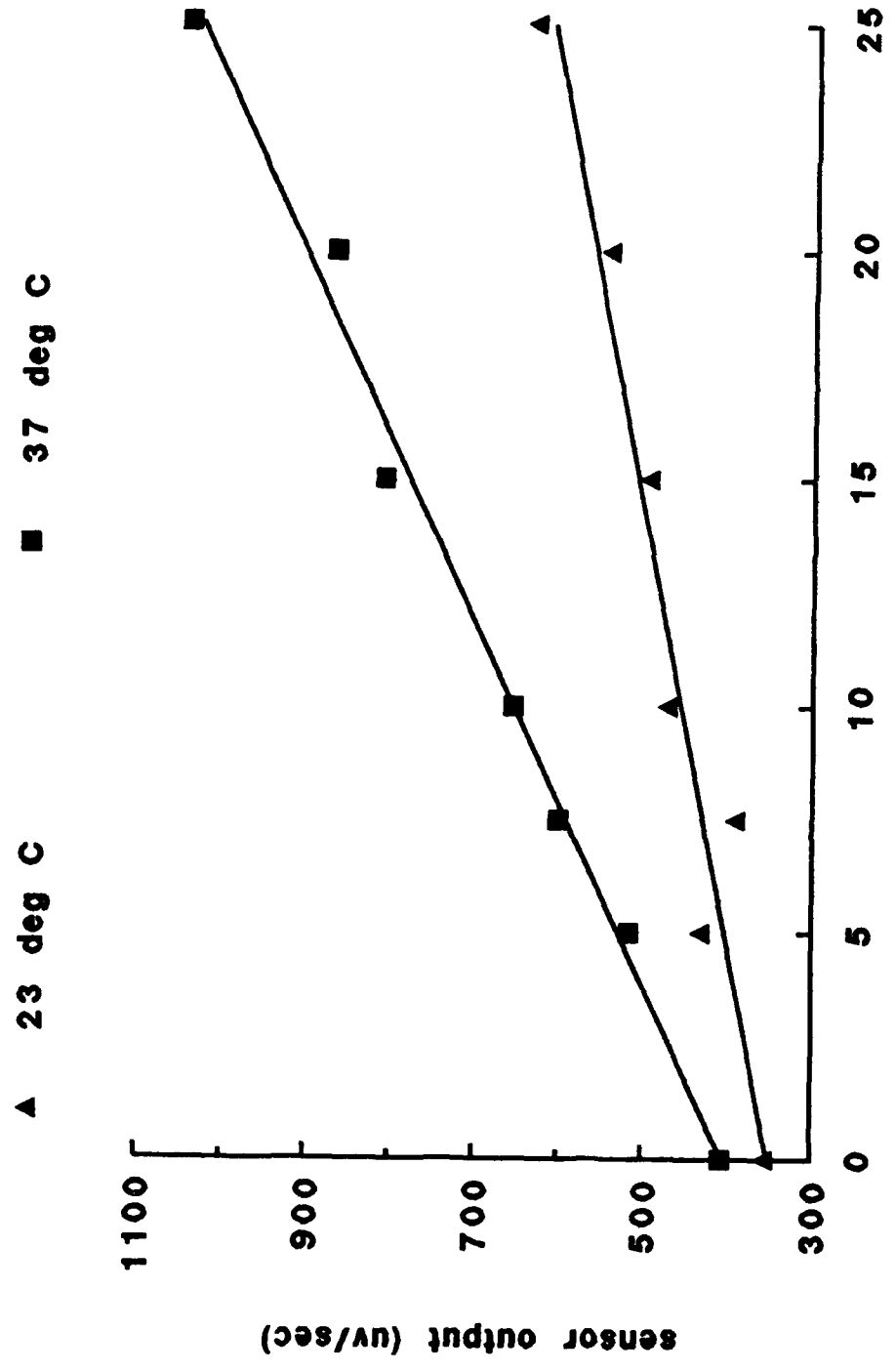

Figure 8

1 MINUTE INCUBATION OF mIgG AT 23°C AND 37°C. THE DATA WERE FITTED BY LINEAR REGRESSION, 23°C, $y = 352 + 10.5x$, $r^2 = 0.967$; 37°C, $y = 404 + 25.0x$, $r^2 = 0.996$.

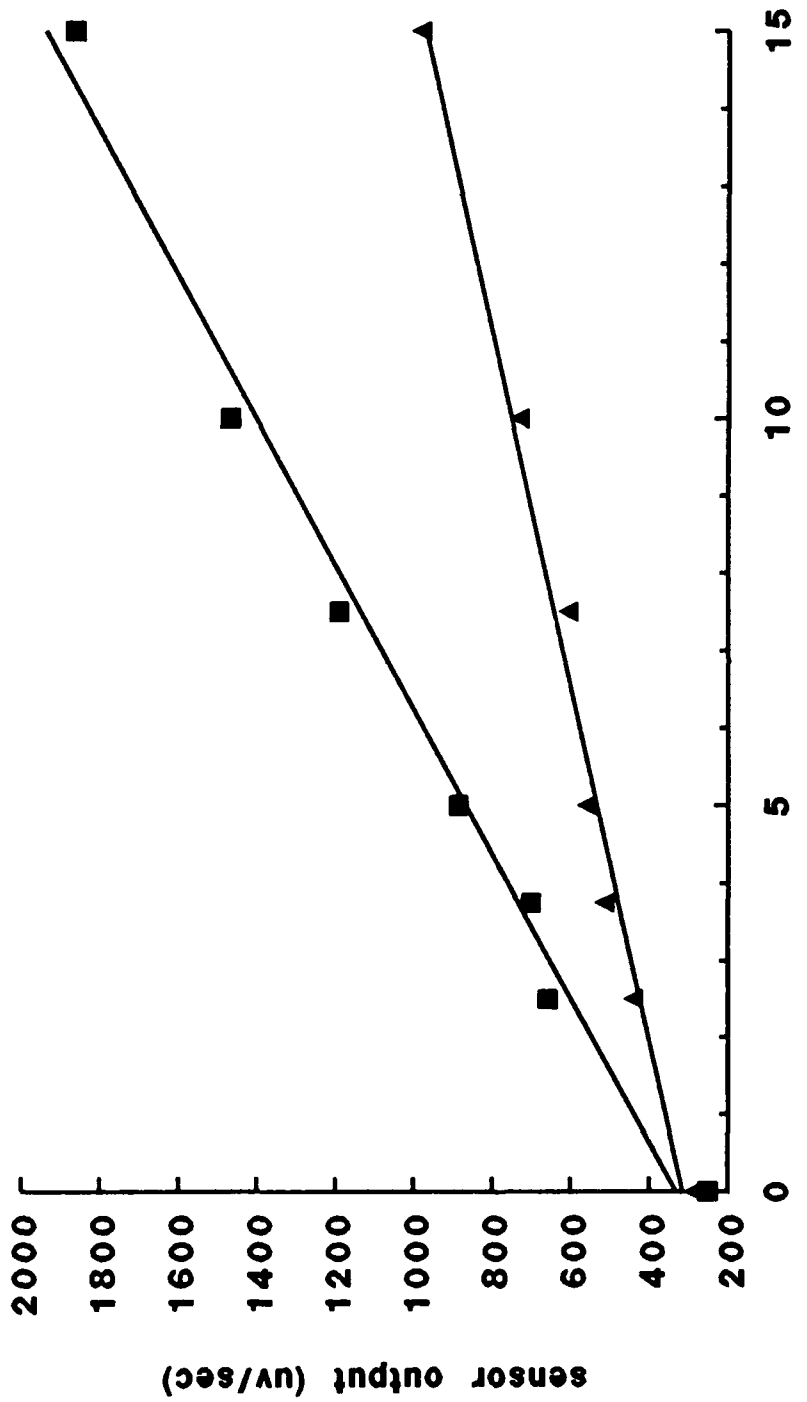

Figure 9

5 MINUTE INCUBATION OF mIgG AT 23°C AND 37°C. THE DATA WERE FITTED BY A LINEAR REGRESSION, 23°C, $y = 317 + 43.6x$, $r^2 = 0.992$; 37°C, $y = 333 + 107x$, $r^2 = 0.993$.

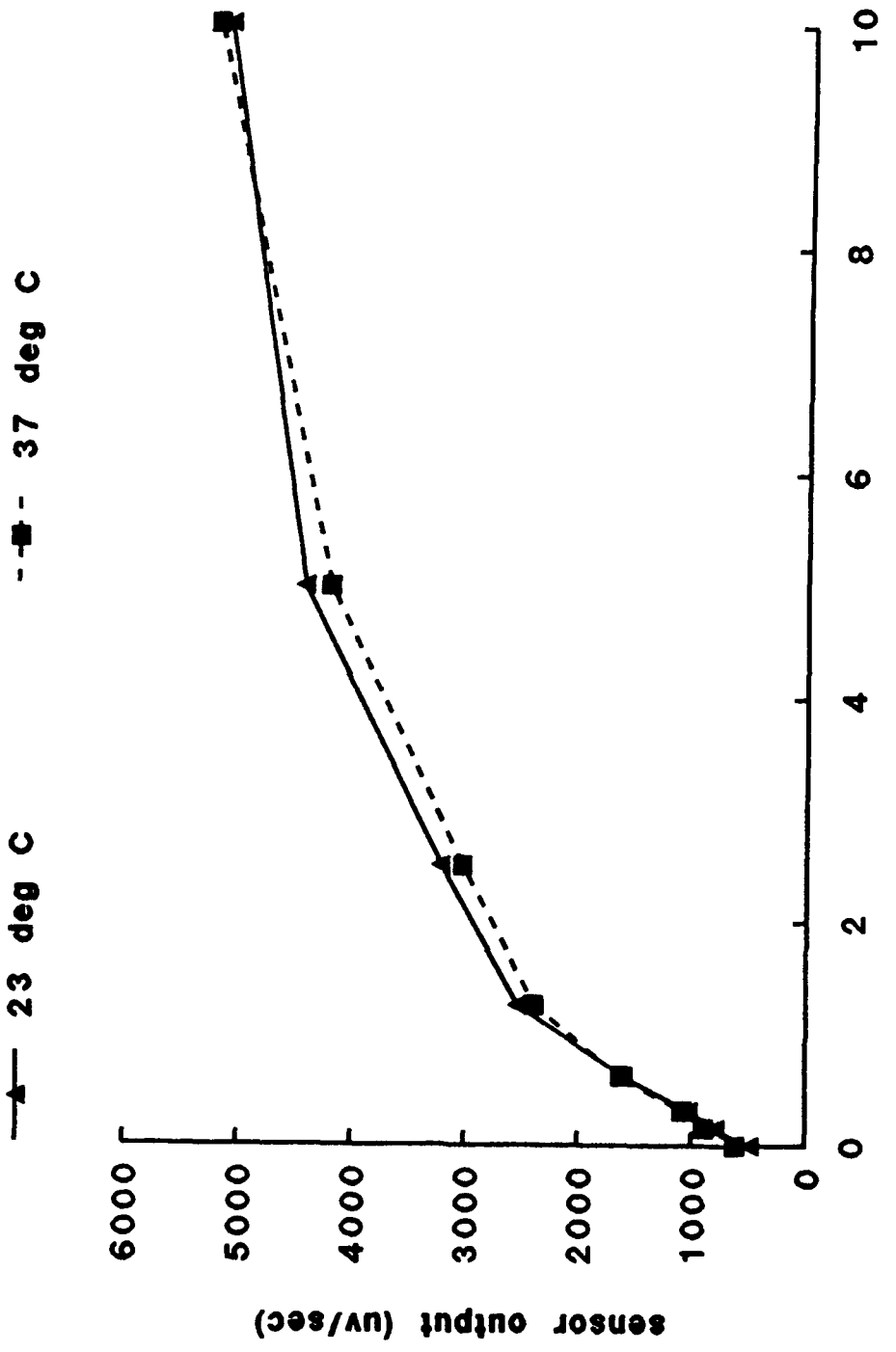

Figure 10

60 MINUTE INCUBATION OF mIgG AT 23°C AND 37°C.

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Immunoassay of Mouse Immunoglobulin G by a Light-Addressable Potentiometric Sensor

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A sensitive enzyme immunoassay for the quantitation of mouse immunoglobulin G (mIgG) was developed using a light-addressable potentiometric (LAP) sensor as the detection system. The assay was carried out on nitrocellulose membrane filters and used sandwich-format one-step incubations of antigens and antibodies. In addition, the assay employed a high affinity biotin-streptavidin interaction to capture the immunocomplexes onto nitrocellulose membranes, and a urease-conjugated anti-mouse IgG detector antibody. The lower limits of detection of mIgG by this assay were 300, 100 and 10 pg per well, respectively for 1, 5 and 60 min incubations. The time required for the assay was the incubation time plus 4-5 min, in total, for the mixing, washing and detection procedures.

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Immunoassay, ELISA, Immunoglobulin

Streptavidin

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